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Integration of Species Sensitivity and Dosimetry Data in the Extrapolation of Ozone and Nitrogen Dioxide Health Data from Animal to Man

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16. ABSTRACT

The integration of species sensitivity and dosimetry data in the extrapolation of ozone (O_3) and nitrogen dioxide (NO_2) health data from animal to man, J. A. Graham and G. E. Hatch, Health Effects Research Laboratory, MD-82, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711.

Estimations of regional pulmonary doses of O_3 and NO_2 need to be combined with an understanding of the sensitivity of several animal species and man to equivalent tissue doses before animal to man quantitative extrapolations can be performed. Because of potential species differences in anti-oxidant defenses and repair mechanisms it is unlikely that regional lung dose-response effects will be identical across species. These differences will be discussed and unifying concepts presented that are likely to quantitatively accomodate the differences in eventual extrapolation modeling. The concepts and their current state of experimental or theoretical validation to be addressed include the application of 1) scaling principles between species following in vitro and in vivo exposure, 2) the parallelogram concept which directly relates acute animal to acute human effects and acute animal to chronic animal effects, thereby permitting the indirect estimation of chronic human effects, and 3) species comparisons of the health effects of O_3.

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Introduction

The goal of animal toxicology studies is to estimate the toxicity of chemicals in man and to define the mechanisms of toxicity. Extrapolation of data from animal to man has always been implied. However, at present, only a qualitative extrapolation is possible. If a pollutant causes an effect in several animal species, most toxicologists would agree that it is likely to cause the same type of effect in man, albeit at an unknown exposure level. Such qualitative extrapolations have been used indirectly in the risk assessment and risk management processes in setting National Ambient Air Quality Standards (NAAQS). Studies in animals are the only research approach that can provide cause-effect data under controlled and defined conditions with virtually unlimited exposure conditions (including chronic) and methods enabling a relatively complete description of a very broad array of reversible or irreversible toxic effects. For scientific purposes, it is essential to go beyond the qualitative extrapolation. In risk assessment we must know, with a reasonable degree of certainty, at what concentration man might experience a toxic effect observed in animals. Such quantitative extrapolation is essential before an informed judgment can be made about the full array of potential human health effects. To perform quantitative extrapolation, three major categories of needed information have been identified: dosimetry, species sensitivity, and health effects.[1] Dosimetry refers to determining the amount of pollutants which reach specific sites in animals and man after exposure to a given concentration of pollutant (e.g., tissue dose resulting from exposure to a given concentration of pollutant). However, even when equivalent doses are delivered to these target sites, there is a possibility that the responses may differ among species. Thus, species sensitivity data are needed, and dosimetry and sensitivity results need to be linked to achieve the quantitative extrapolation of effective pollutant concentrations between animals and man. Even when quantitative extrapolation for this discussion becomes possible, there must be an adequate understanding of health effects from which to extrapolate. To focus on the topic of extrapolation, ozone (O_3) and nitrogen dioxide (NO_2) have been chosen, and O_3 will be emphasized as a test case since models of regional lung dosimetry of these gases are reasonably advanced and have a more advanced health data base for animal and human studies. Also, the toxicology of O_3 has several elements that facilitate species sensitivity studies, making it the NAAQS pollutant most likely to be extrapolated in the future. In this Symposium, Overton and Miller[2] will discuss lung dosimetry of O_3 and NO_2, and Smolko et al.[3] will describe applications of the O_3 dosimetry model and state of the health data base to be extrapolated. This paper will discuss the remaining elements of extrapolation - species sensitivity and approaches to linking sensitivity to dosimetry. The limited data available will be presented along with approaches that could be used to provide the needed information.

Species Sensitivity to Ozone

Comparisons of the effects of O_3 on different animal species is of interest in attempting to understand whether or not man might experience similar effects. For example, if only one of several tested species

experienced a given effect of O_3, this effect might be species specific and not occur in man. Conversely, if several animal species, with all their inherent differences, shared a given effect of O_3, it would be likely that a common element present in all mammalian species, including man, was susceptible to O_3. To detect a commonality of effects across species would be expected, provided the effect was related to a mechanism which is shared across species. In the case of O_3, the proposed major molecular mechanisms of action are the oxidation of polyunsaturated fatty acids and the oxidation of thiols or amino acids.[4,5] Thus, the affected molecules are identical across all species. Any differences in the observed responses between species would thus be a function of species differences in delivered doses or of subsequent processes of toxicity. For example, the carbon-carbon double bond would be affected equally across species, but the consequent toxic impact on the membrane of a Type I cell could be influenced by species differences in antioxidant defenses or repair mechanisms. Since current knowledge is not sufficiently advanced to assess such events quantitatively, only qualitative hypotheses can be made based on the available evidence. A commonly accepted hypothesis is that if O_3 causes an effect in several animal species, it can cause a similar effect in man. It is important to note that this is a qualitative probability; it does not permit assessment of the concentrations at which man might experience the common effect. Indeed, from dosimetry studies,[6] it is likely that different concentrations would cause effects across species (including man).

The health data base for O_3 consists of hundreds of studies with about 7 species, and even more strains, of animals. Generally, for a given effect, whether it be on lung morphology, physiology, biochemistry, or host defenses, all species tested were responsive to O_3, albeit sometimes at different concentrations. Unfortunately, to move from this general comment to a more specific quantitative analysis is not possible since multiple species studies using identical techniques, exposure patterns, and concentration-response designs are required. Only one such study has been reported. With this in mind, the few O_3 studies of several species having at least two points of identity for comparison will be discussed.

Morphological examinations of the lungs of several animal species have been conducted after O_3 exposure. Of the animals studied, there are significant differences in lung structure. Humans, nonhuman primates, and dogs have respiratory bronchioles, while other common laboratory animals have either poorly developed ones or none at all. Additional differences exist. Nonetheless, a characteristic O_3 lesion occurs at the junction of the conducting airways and the gaseous exchange tissues, regardless of the species differences in the structure. The typical effect in all the species examined is damage to ciliated and Type I cells and hyperplasia of nonciliated bronchiolar and Type II cells. An increase in inflammatory cells (primarily alveolar macrophages) is also observed. Such changes were observed after a 7-day intermittent exposure of monkeys and rats to 0.2 ppm.[7-9] With different exposure regimens, similar effects occur in cats (.26 ppm, endotracheal tube exposure, about 6 hr),[10] mice (0.5 ppm, 35 days of exposure)[11], and guinea pigs (0.5 ppm, 6 mo).[12] For these studies, lower concentrations of O_3 were not tested. Insufficient data are available to quantitatively

compare the monkey and rat studies mentioned above. Nonetheless, a rough equivalency of responses was observed under a variety of exposure conditions between species having major structural differences. Since there is a relatively slight structural difference between the lungs of monkeys and man, the possibility is enhanced that man would experience similar effects.

Pulmonary function measurements on 5 species of animals has been studied after acute or prolonged O_3 exposure, but there are insufficient points of identity in the experiments to permit direct comparisons. For effects of acute exposure to O_3 on airway reactivity, sheep[13] and dogs[14] may be compared. For both species, exposures were for 2 hr. In sheep exposed to 0.5 ppm O_3, response (pulmonary resistance) to the bronchoconstrictor, carbachol (an analog of acetylcholine), was increased 24 hr post-exposure; at 1 ppm O_3, response was increased immediately and 24 hr post-exposure. In dogs exposed to 0.7 to 1.2 ppm O_3, the pulmonary resistance increase due to histamine (a bronchoconstrictor) was observed at 24 hr, but not 1 hr, post-exposure. Humans also exhibit an increased responsiveness to the bronchoconstrictors histamine, methylcholine (an analog of acetylcholine) and acetylcholine immediately after a 2 hr exposure to $\geq$ 0.4 ppm O_3.[13-14] One day post-exposure, this increased responsiveness was not observed in humans.[17]

Species comparisons of host defense against infection are possible using alveolar macrophages. After an intermittent (8 hr/day) 7 day exposure to 0.2 ppm O_3, an increased number of alveolar macrophages was observed in the lungs of both rats and monkeys.[7-9] Although numerous other macrophage studies have been conducted, there are insufficient points of identity for species comparisons.

Rats and monkeys have been examined for changes in lung biochemistry following O_3 exposure. In these animals exposed for 7 days (8 hr/day) to 0.8 ppm O_3,[18] glucose-6-phosphate dehydrogenase activity in whole lung homogenates was elevated to a roughly equivalent degree. Glutathione reductase activity was increased in rats, but not monkeys. Chow et al.[19] also compared these species after exposure to 0.5 ppm O_3 for 8 hr/day for 7 days. Antioxidant enzymes were increased in the rats, but not the monkeys. The authors referred to "relatively large variations" in the monkey data. Oxygen consumption was measured in rats and monkeys after a 7 day (8 hr/day) exposure to several levels of O_3.[20] Rhesus monkeys may have been slightly less responsive than rats. However, at 0.5 ppm O_3, bonnet monkeys and rats had roughly equivalent increases. In all of these reports, there was no mention of statistical comparisons between species or of power calculations that would indicate whether under the experimental conditions of data variability, there was equivalent power to statistically detect effects in both species. In a few of the reports, the number of animals was not given. Mustafa et al.[21] compared mice to 3 strains of rats exposed to 0.45 ppm O_3 continuously for 5 days. Antioxidant metabolism and oxygen consumption were measured. Generally, increases in enzyme activities were observed in both species; in several cases the increase in the mice was statistically greater than the increase in the rats.

Hatch et al.[22] have performed the only reported experiment (other than mortality studies) in which several species of animals were identically exposed to several levels of O_3 and identically examined. Mice, hamsters, rats, guinea pigs, and rabbits were exposed for 4 hr to 4 levels of O_3 (0.2, 0.5, 1.0, and 2 ppm) and protein content of the lung lavage fluid was measured as an index of edema 16 hr post-exposure. All species exhibited a trend of increasing protein content as O_3 concentration increased. Guinea pigs and rats appeared to be more responsive. For example, guinea pigs were affected at 0.2 ppm and rats at 0.5 ppm. Other species only had an increase in lavage fluid protein at 1 or 2 ppm O_3.

To adequately assess species sensitivity to a given delivered dose of O_3 or NO_2, mechanisms which might influence the sensitivity must be understood better. Only recently is such information becoming available. The lungs have several defense mechanisms against oxidants. Dietary, and presumably, constitutive vitamin E protect against O_3 and NO_2 toxicity.[4,23] Vitamin C appears to protect against NO_2, but not O_3, toxicity.[24,25] Nonprotein sulfhydryls and several enzymes (e.g., superoxide dismutase, catalase, and the glutathione peroxidase) are also involved in antioxidant defenses. If basal levels of these defenses differed across species, there would be a potential difference in species sensitivity to oxidants. Using this as a hypothesis, Slade et al.[26] measured vitamins C and E and nonprotein sulfhydryls in the lungs of mice, hamsters, rats, guinea pigs, rabbits, domestic pigs and sheep and humans (samples from cancer surgery patients). Average lung levels of vitamin C were roughly equivalent for hamster, rabbit, sheep, pig, and man. Rat and guinea pig levels were slightly higher. Mice had considerably higher levels of vitamin C than hamsters (42 mg% vs. 25 mg%). For vitamin E, domestic pigs had the highest levels (about 3.7 mg%) followed by rats (2.7 mg%) and the other species. Man had lung vitamin E levels of about 0.9 mg%. Human cancer patients had levels of nonprotein sulfhydryls that were quite low (about 5 mg%), possibly due to anesthesia, or drug therapy. Mice, hamsters, and rats ranged from 50-65 mg%, whereas guinea pigs and rabbits had about 80 mg% nonprotein sulfhydryls. Thus, differences in basal lung levels of vitamins C and E, and non-protein sulfhydryls were observed among the species. However, these differences in lung antioxidants did not appear to correlate with differences in sensitivity of the species to the edemagenic effects of O_3 observed in the previously mentioned study.[22] It should also be noted that neither the differences in antioxidant levels nor the differences in *in vivo* sensitivity to O_3-induced edema appeared to be that remarkable in any case. Most species fell within a 2-fold range, therefore, large differences in tissue sensitivity based on basal antioxidant metabolism among species should not be expected.

For extrapulmonary effects, the only species comparison using identical methods was made by Graham et al.[27] Female mice, rats, and hamsters had an increase in pentobarbital-induced sleeping time after a 5 hour exposure to 1 ppm O_3. Under the experimental conditions used, relative species responsivity cannot be assessed.

An overview of the animal toxicological data base for O_3 indicates that rats are the most prevalent species tested. Other species often

used include mice, rabbits, guinea pigs and monkeys. A few dog and hamster studies exist. As has been noted above, very few species comparisons can be made due to differences in exposure regimens and measurement techniques. Even when direct comparisons are possible, interpretation is difficult. Statements regarding responsiveness can be made. Statements about sensitivity (e.g. responses to an equivalent delivered dose) cannot be made until more dosimetry and other types of data are available. Nonetheless, it is remarkable that even with the wide variation in techniques and experimental designs, acute and subchronic exposures to levels of O_3 less than 0.5 ppm produce several types of responses in many species of animals. Thus, it may be hypothesized that man will experience more types of effects than those established in human clinical studies. Types of effects, for which a substantial animal data base exists, include changes in lung structure, biochemistry, and host defenses. However, the actual risk to man breathing ambient levels of O_3 cannot be determined until the animal data can be quantitatively extrapolated to man.

Development of Quantitative Linkages Between Experimental Studies and Potential Human Effects

Even when the appropriate bases for quantitative extrapolation are created, they must include components that will provide internal linkages to correlate dosimetry, sensitivity, and health studies, if extrapolation is to be realized. Thus, here we describe general considerations that need to be applied to actual research studies as well as concepts that need to be included in extrapolation research.

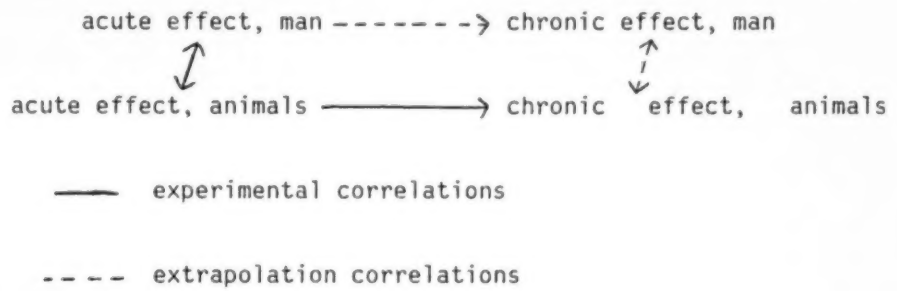
By using a combination of experimental and theoretical studies, the regional pulmonary doses of O_3 and NO_2 can be determined in laboratory animals and man under acute exposure conditions. It will then become necessary to evaluate dosimetry under chronic exposure conditions, a more difficult undertaking because there may be toxicological changes that will have a dynamic influence on dosimetry. For example, the morphometry of the lungs is a factor in dosimetry models. Ozone alters morphometry. However, it is unknown whether this alteration will affect dosimetry. Human chronic dosimetry experimental studies cannot be conducted for ethical reasons, making reliance on animal studies imperative.

Similar issues arise when considering species sensitivity. To a limited degree, equivalent acute exposure experiments in animals and humans can be performed. However, invasive methods, which provide a fuller understanding of toxicity and a better definition of risk, cannot be performed in man, even under acute exposure conditions. Similarly, chronic studies, which are known to cause irreversible health effects, cannot be performed in man. How then can linkages be created to enable extrapolations?

The development of such linkages is at the heart of experimental medicine, and the research to develop methods defining such linkages will help focus future research needs. A basic goal has been to define

fundamental physiological, anatomical, and biochemical mechanisms leading to the toxic lesion(s). The rigor of such an effort will increase the degree of confidence that can be placed on eventual judgments concerning human health. Several such concepts described in the following need to be studied, integrated, and applied to the extrapolation of health effects in man. For example, a specific approach for evaluating host lung defenses could not necessarily be applied precisely to measurements of pulmonary function. In addition, no single approach will be fully useful for a given endpoint.

An approach to linking acute and chronic effects is by a parallelogram concept, as described below. For several types of effects, including those on lung function, alveolar macrophages, airways epithelium, surface liquid composition, and mucociliary clearance, acute concentration-response exposure studies could be performed in man and in several species of animals. The experimental documentation of the transition of acute injury to chronic effects would be made in animals, because controlled chronic exposures in humans are not possible. Integration with other principles described in other subsections would assist in the development of the extrapolation relationships between chronic animal and human effects, thereby completing the parallelogram shown below.



Although human studies are often considered to be severely limited by obvious ethical considerations, the scope of non-invasive or minimally invasive approaches to investigation of mechanisms by which toxic agents exert their effects has increased dramatically. Some measures of toxicity, in particular effects on alveolar macrophages, lend themselves to an approach involving integration of *in vitro* and *in vivo* dose-response relationships. Using *in vitro* exposure methods, alveolar macrophages of several animal species and man could be exposed to pollutants under conditions in which actual cellular dose could be determined. From such dose-response curves, a quantitative estimation of species sensitivity can be obtained. These same species (including man) could then be exposed *in vivo*, alveolar macrophages isolated from bronchioloalveolar lavage liquid, similar endpoints measured, and exposure concentration-response curves developed. By using dosimetry calculations based on previously developed models, the exposure concentration could be transformed to site-specific dose. The specific *in vivo* and *in vitro* relationships could be quantitatively evaluated. The relative placement of man in each system and the relation of *in vitro* to *in vivo* exposure effects would be known. This would allow the sensitivity of man to be estimated in relationship to other species.

One of the most useful evaluations of toxicity uses morphometric techniques that allow quantification of structural and cellular alterations. Since lung tissue is required, this technique cannot be applied to humans, whether the exposure is acute or chronic. Fortunately, structure, function, and biochemistry are known to be interrelated, even though more precise correlation studies will be required for extrapolation. The general approach in this area would again utilize the parallelogram concept and determine scaling factors for endpoints suitable for human experimentation (e.g., changes in pulmonary function, alveolar macrophages, or epithelial barrier function). In animals, these endpoints could be experimentally related to morphometric alterations. These structural changes in animals, when related to other alterations (i.e., pulmonary function, etc.) observed experimentally in animals and humans, would enable extrapolation to potential structural changes in man. Understanding the concentration-effect and the initiation or acceleration of human disease is central to this approach. Description of early changes in animals leading to chronic disease may lead to similar observations in man. Thus, predicted effects may be detected in man through clinical experience.

Summary

Quantitative extrapolation of animal data to man is essential if all the major effects of O_3 and NO_2 are to be considered directly in the standard setting process. For example, animal data show that these pollutants cause major structural alterations in the lung, involving chronic lung disease. The inherent nature of human clinical studies and epidemiology is such that these types of effects cannot be proven, even if they exist. Through quantitative extrapolation the risk to man of chronic lung disease upon exposure to oxidants should be possible to estimate on a scientific basis. Achieving such a goal will require a much greater knowledge of the components of extrapolation and the linkage of these components than is presently available. If the coordinated expertise of the research community is brought to bear on the topic, its achievability is inevitable.

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